Original Article

Study of thalassemias and hemoglobinopathies in pregnant females in rural areas of north western India

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ABSTRACT

Background: Anaemia during pregnancy is a major contributing factor to childhood anemia. The most common cause of anaemia in pregnancy is nutritional anemia. When anemia of pregnancy becomes unresponsive to therapeutic measures, workup for other causes of anemia needs to be done. Hemoglobinopathies and thalassaemias are the most common single gene disorders in the world. Worldwide, the Hb disorders are responsible for 3.4% mortality in children below 5 years of age. High performance liquid chromatography (HPLC) is a simple and rapid method of detection of different Hb variants. The knowledge of the frequency of common Hb variants encountered in pregnant females in a particular area is important for the formulation of specific diagnostic, preventive and therapeutic strategies. There has been no study documenting the prevalence of hemoglobinopathies in pregnant females of Rajasthan, India.

Aim: The aim of the present study was to determine the common Hb disorders in pregnant females of a tertiary care hospital of Rajasthan, India.

Material & Methods: The study was carried out in India with participants recruited during an antenatal care visit made to participating community health and primary health centres (CHCs and PHCs) in the state of Rajasthan. This study was conducted in the Advanced Hematology & HLA Laboratory, Department of Pathology of a tertiary care center.

Results: A total of 1851 pregnant females had their blood samples collected out of which 500 females' samples were evaluated for HBA, HBA2 & HBF by cyanomethemoglobin method. 51 females out of these 500 tested positive for hemoglobinopathy (10.20%). Out of the 51 hemoglobinopathies, 49 were Beta Thalassemia heterozygous, one HPFH & one HBD Punjab.

Conclusions: The most common Hb abnormality detected in this study was that of β thalassemia heterozygous (9.8%). Due to the high prevalence of Hb disorders in various regions, the premarital screening must be routinely done for prevention of high-risk marriages.

INTRODUCTION

Anaemia during pregnancy is a major contributing factor to childhood anemia. The most common cause of anaemia in pregnancy is nutritional anemia. When anemia of pregnancy becomes unresponsive to therapeutic measures, workup for other causes of anemia needs to be done.

Hemoglobinopathies and thalassaemias are the most common single gene disorders in the world.[1] Thalassaemias are characterized by reduced synthesis of one or more globin chains of the hemoglobin (Hb) molecule while hemoglobin variants are a result of production of normal amounts of mutant globin chains.[1] World Health Organization figures estimate that 7% of the world populations are carriers of a potentially pathological hemoglobin (Hb) gene and every year 60000 thalassaemia babies are born all over the world.[1]

The prevalence of thalassaemias and hemoglobinopathies varies with geographic locations. It has been estimated that in India, 0.37/1000 fetuses have a Hb disorder.[2] Worldwide, the Hb disorders are responsible for 3.4% mortality in children below 5 years of age.[3]

High performance liquid chromatography (HPLC) is a simple and rapid method of detection of different Hb variants. Clinical history and findings of thorough hematologic evaluation, including complete blood count, reticulocyte count and red blood cell morphology are necessary to reach an accurate diagnosis. Family studies are often required to detect a particular Hb variant.[8]

The knowledge of the frequency of common Hb variants encountered in pregnant females in a particular area is important for the formulation of specific diagnostic, preventive and therapeutic strategies. There has been no study documenting the prevalence of hemoglobinopathies in pregnant females of Rajasthan, India. The aim of the present

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study was to determine the common Hb disorders in pregnant females of a tertiary care hospital of Rajasthan, India.

METHODOLOGY

The study was carried out in India with participants recruited during an antenatal care visit made to participating community health and primary health centres (CHCs and PHCs) in the state of Rajasthan.

Inclusion criteria for screening and consent were as follows: (1) pregnant women between 18 and 40 years of age and capable of giving informed consent; (2) Hb concentration of 7–10.4 g/dL (as per the available point of care laboratory report).

This study was conducted in the Advanced Hematology & HLA Laboratory, Department of Pathology of a tertiary care center. A signed consent form was obtained from all the participants included in the study. A detailed clinical history and family history were obtained from each patient. Blood samples were collected in ethylene diamine tetrachloride acetate vials and analyzed with Sysmex automated cell counter (XN 1000) for complete blood counts.

SAMPLE SIZE:

The sample size was calculated at 95% confidence level assuming 4.61% hemoglobinopathies among pregnant patients as found in reference study[3] at the precision of 2% (absolute allowable error). Minimum 433 pregnant women were required as sample size which is enhanced and rounded off to 500 pregnant females expecting 15% attribute.

Sample technique:

11 PHC AND 5 CHC of Jaipur CMHO I and CMHO II were selected randomly and 500 pregnant women were taken according to probability proportionate to size method to make sample representative of Jaipur district.8

Material and methods

The tests were performed by high performance liquid chromatography (HPLC) based upon the principles of cation exchange chromatography using the BIORAD VARIANT TM Hb typing system (Variant Beta-Thalassemia Short program) (Biorad laboratories, California, USA). A Hb A2/F calibrator were analyzed at the beginning of each run. The different Hb variants like HbE, S, D and C were identified by using retention time windows, that was defined as the time in minutes from sample injection to the maximum point of the elution peak, which are specified for each of these variants. Normal adult chromatogram shows primarily HbA (97-98%), a small percentage of HbA2 (<3.5%) and traces of fetal Hb (<1%).

The data were analysed using Microsoft excel 2010 version.

RESULTS

A total of 1851 pregnant females had their blood samples collected out of which 500 females' samples were evaluated for HBA, HBA2 & HBF by cyanomethemoglobin method. 51 females out of these 500 tested positive for hemoglobinopathy (10.20%)

Prevalence of haemoglobinopathies

Haemoglobinopathies	No.	%
Present	51	10.20
Absent	449	89.80
Total	500	100.00

Out of the 51 hemoglobinopathies, 49 were Beta Thalassemia heterozygous, one HPFH & one HBD Punjab Trait. The distribution of different Hb patterns in the study population has been shown in **Table 2**. For each of these groups, the percentage of various Hb detected in HPLC have been shown in **Table2**. Interpretation of results of HPLC was done on the basis of retention time, percentage of Hb and peak characteristics.

Prevalence of various types of haemoglobinopathies

Types of haemoglobinopathies	No.	%
Hb D Punjab Trait	1	0.20
HPFH	1	0.20
β Thalassemia heterozygous	49	9.80
Normal	449	89.80
Total	500	100.00

Parameters	Hbpathies	N	Mean	SD	Median	Min.	Max.	'p' value*
HB	**Present**	51	9.51	1.06	9.4	7	12.3	0.285
	Absent	449	9.28	1.49	9.4	4.2	13.1	
MCV	**Present**	51	65.11	5.46	64.4	55.1	78.4	<0.001
	Absent	449	74.67	6.52	75.1	56.8	96.9	
MCH	**Present**	51	20.11	1.99	19.8	15.9	24.4	<0.001
	Absent	449	22.83	3.07	23	13.3	32.6	
MCHC	**Present**	51	30.84	1.00	31.1	28.7	33.4	0.207
	Absent	449	30.51	1.83	30.7	24.3	34.8	
RET He	**Present**	51	20.92	2.59	20.9	15.2	27.4	<0.001
	Absent	449	23.19	4.72	23.2	12.2	36.5	
Hb By Cynmet hemoglobin	**Present**	51	8.42	1.10	8.2	6	10.9	0.212
	Absent	449	8.17	1.37	8.2	3.4	12	
HbA	**Present**	51	82.27	4.60	83.4	54.9	85.2	<0.001
	Absent	449	86.06	1.64	86.3	70.9	88.8	
HbA2	**Present**	51	5.19	0.89	5.3	0.9	6.6	<0.001
	Absent	449	2.60	0.36	2.6	0.5	6	
HbF	**Present**	51	1.49	2.40	0.9	0.3	17.3	<0.001
	Absent	449	0.45	0.30	0.4	0	4.1	

*Independent Sample 't' Test

This table compares hematological parameters of pregnant females with & without hemoglobinopathies. Significant differences have been found in MCV, MCH, Reticular Hb, HBA, HBA2 & HBF

DISCUSSION

Haemoglobinopathies pose a major health problem in India especially during pregnancy. It has been estimated that in India, 0.37/1000 fetuses have a Hb disorder.[1] The data on the prevalence of β-thalassemias and other haemoglobinopathies in pregnant females of India is scarce. Therefore, the present study was undertaken to determine the prevalence of haemoglobinopathies in different caste/ethnic groups using uniform methodology.

Most prevelant hemoglobinopathies include beta thalassemia, HbE, HbD and sickle cell anemia. The

prevalence of beta thalassemia mutations is as high as 17% in some Indian populations. The prevalence of HbD in our country is estimated to be approximately 1.1%.[2]

High performance liquid chromatography (HPLC) is a simple and rapid method of detection of different Hb variants. Clinical history and findings of thorough hematologic evaluation, including complete blood count, reticulocyte count and red blood cell morphology are necessary to reach an accurate diagnosis.

In the present study, the prevalence of Hb disorders in pregnant females of rural India was found to be 10.2%. A study conducted in the southern part of West Bengal has reported a higher prevalence (25%) of thalassemias and hemoglobinopathies.[3] a study conducted in north Indian population has reported an incidence of hemoglobinopathies to be 12.5%.[4] The prevalence rate of Hb disorders was reported to be 7% in Bhopal.[5]

The most common Hb abnormality detected in this study was that of β thalassemia heterozygous (9.8%). Colah et al. reported that nearly 1.5% of the world's population is carriers of β thalassemia. [6] In rural Bengal, the prevalence of β thalassemia trait has been reported to be as high as 10.38%.[7] In central India, the prevalence of β thalassemia trait has been estimated to be 9.59%.[8] However, in Orissa, sickle cell trait was the most common abnormality found.[15] In the present study, sickle cell trait was found in 0.46% cases.

In this study, HbE trait was found in 2.68% cases and Eβ thalassemia in 1.56% patients. A study conducted in the rural areas of West Bengal reported the prevalence of HbE trait to be 3.86% and that of Eβ thalassemia, 1.25%. [16] Due to the high prevalence of Hb disorders in various regions of West Bengal premarital screening must be routinely done for prevention of high-risk marriages.[17]

Other variants detected in the present study included HPFH.

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